METHODS & TECHNIQUES

Preserving Terrestrial Slugs by Freeze-Drying

BY

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Terrestrial slugs (families Limacidae, Arionidae, and others of the molluscan class Gastropoda) are usually preserved for study in alcohol or formalin solutions. Both of these preservatives deprive the specimens of much of their natural color, resulting in very unattractive displays. Furthermore, "wet" collections have the disadvantage of occupying more space and of requiring more maintenance than do "dry" collections. The process of freeze-drying was investigated, with the objective of developing a preservative securing more natural appearing specimens which could be stored and displayed easily as synoptic collections or teaching aids.

It soon became apparent that there were several independent steps to the process which were essential for producing acceptable specimens. These are as follows:

(1) the slug must be inactivated (or killed) in the natural extended position desired in the final product;
(2) frozen in the desired posture;
(3) freeze-dried (a process requiring a specialized piece of equipment);
(4) coated with a clear shiny material to bring out the color and markings of the specimen and to give it the appearance of a moist mucus coating; and finally
(5) mounted as a specimen for display or study.

It should be pointed out that freeze-dried slugs are probably not good material for serious taxonomic studies, since diagnostic characters in this group of organisms rely heavily on form and arrangement of various parts of the reproductive system and on the position of major muscle groups.

[1] Oregon Agricultural Experiment Station, Technical Paper No. 3275

Drowning

Possibly the most important step in the freeze-dry process with slugs is the first – the immobilization of the specimen in a natural position for freezing. The time-honored method of killing slugs for preservation is by drowning in water. Because of the heavy secretion of mucus in the presence of irritating chemicals, most methods for killing and fixing small animals are not applicable to slugs. A drowned slug is usually well extended, but one must position the animal carefully before freezing to prevent dents, creases or other unnatural deformations.

A small slug can be drowned in a tightly capped container full of water at room temperature in about 12 hours. Almost 24 hours are required to drown a large specimen, such as a mature *Arion ater*. A "half-drowned" slug may appear dead, but will later contract into an undesirable position when placed in the freezer. An "over-drowned" slug decomposes rapidly.

The desired situation is to render the extended animal completely immobile so that it can be frozen in a natural posture within a reasonable length of time. Since the use of sugar, thymol, chloral hydrate, and other additives to the water used for drowning have been suggested by various workers for improving the appearance of slugs being readied for preservation, we studied the use of several drugs and other chemicals, hoping to reach the right state of immobility of the animals quicker and with more dependability than by simple drowning.

Use of Drugs, Salts, and other Chemicals

The test summarized here involved use of chemicals as additives to immersion water, and the results are given in Table 1. Some potential materials, such as Chloretone (Hubricht, 1951), were not tested in this rather cursory study. Propylene phenoxetal (Rosewater, 1963) used alone was not satisfactory, but the combination with Nembutol, described by Runham (1965) as being rapidly effective against land slugs, was not tested.

Of the various treatments recorded in Table 1, none gave the results desired except ethyl alcohol. This treatment suggested itself because of the current popularity of using dishes of beer in home gardens for control of slugs (Smith, 1970). Beer can substitute for the 5% alcohol dilution as a narcotizing agent.

An exposure time of 1½ hours in 4 - 6% ethyl alcohol is a minimum for large slugs to prevent them from moving after being put in the freezer. More studies are needed to determine how short an exposure would be necessary for

immature forms or small species. Since over-night to 24 hour drowning is required with plain water, the use of alcohol greatly shortens the time needed to process a slug for freezing.

Freezing

Once the animal is completely immobilized, it should be positioned in a container (such as a Teflon-coated pie pan) which can later be placed in the lyophilizer. Any

Table 1

An annotated list of drugs and salts tested as narcotizing agents on slugs in immersion water solutions

Material and Concentrations of Immersion Solutions	Species tested [1]	Remarks
Benzocaine (0.01 - 0.1%)	*Deroceras* *Arion* *Limax* *Helix*	Ineffective at 0.01%; excessive sliming above 0.025%
Propylene Phenoxetol (3-phenoxypropanol) (0.5%)	*Arion* *Deroceras*	Excessive sliming and shrinkage even after prior exposure to benzocaine
Carbaryl (Sevin) (ca. 1% of 50% W. P.)	*Prophysaon*	Immersion for 24 hours; inferior to specimens drowned in water alone
Nembutol (sodium pentobarbitol) (0.25% - 4.0%)	*Helix* *Arion* *Deroceras* *Limax flavus* *Limax maximus*	Little sliming up to 2 hours in 4%, but animals still reactive to stimuli; quiet and positioned well, but contracted under refrigeration
Nembutol (2 - 4%) DMSO (dimethyl sulfoxide) (4 - 6%)	*Arion* *Helix* *Limax maximus* *Limax flavus*	Positioned poorly; tended to be stiff or excessively flaccid
Sucrose (1.0, 0.5, 0.1%)	*Arion* *Helix*	*Helix* swelled excessively; *Arion* slimed excessively
Salt Solutions:		
NaCl (0.9, 0.7, 0.5, 0.3, 0.1%)	*Arion* *Helix*	Excess sliming down to 0.3%; below this concentration no difference from water
$Ca_3(PO_4)$ [2]	*Arion* *Helix*	Good posture after 6 hours in 0.2 - 0.05%, but excess sliming with *Arion*
MgCl [2]	*Arion* *Helix*	Animals tend to swell excessively
KCl [2]	*Arion*	0.2% conc. gave better appearing specimens than water, but no reduction in time
$Na_3(PO_4)$ [2]	*Arion* *Helix*	Excessive sliming at 0.1%
Ethyl alcohol (4 - 8%)	*Arion* *Limax*	1½ hours in 4 - 6% very satisfactory: minimum sliming, no contraction in freezer; 3% not effective enough; 7% caused excessive sliming. Disadvantage: tentacles not well extended
Beer (ethyl alcohol content ±5%)	*Arion* *Limax*	Results similar to dilute ethyl alcohol. *Limax* tends to be more flaccid in beer

[1] Species tested were *Deroceras reticulatum, Arion ater, Helix aspersa, Prophysaon andersoni,* and *Limax flavus* unless otherwise stated

[2] Various concentrations tested in "effect – no effect" ranges

freezer which can keep ice cream hard is suitable for freezing slugs. If the specimen is to remain more than a day or two in the freezer, it should be covered to prevent dehydration or "freezer burn." Twenty-four hours in a freezer at −12° C or lower is sufficient to freeze a specimen solidly. An attempt to quick freeze a slug specimen not previously drowned or anesthetized, by dropping it into liquid nitrogen, produced a very unsatisfactory specimen. Even at the extremely low temperatures involved, the slug, a mature *Arion ater,* was able to contract its body and secrete a considerable amount of slime!

Lyophilizing

The equipment used successfully in these studies was the Virtis "UNITRAP", Model 10-103 (The Virtis Company, Inc., Gardiner, New York). The vacuum pump and motor do not come with this model and CENCO Hyvac 7 or equivalent is recommended by the Virtis Company for use with their "Unitrap" lyophilizer. Another accessory necessary for bulk freeze-drying of such things as slugs is a "heat rack." Instructions for the operation of the freeze-drier come with the equipment. Twenty-four to 48 hours are required to dry a pan of frozen slugs. The principal precaution to take for this step in the process is to arrange for transfer of the slug specimens from the freezer to the lyophilizer without thawing them to any degree. The specimens must be solidly frozen when they are placed into the readied lyophilizer.

Cleaning and Coating

Specimens taken directly from the lyophilizer have a faded and dusty appearance. This is partly due to the presence of freeze-dried mucus on the surface of their bodies. A camel's-hair brush can be used to remove most of the dry mucus. At this stage the specimen can be handled rather roughly.

To darken the specimen and bring out its natural pigments, we found that any clear coating material, such as shellac, lacquer or plastic preparation, was satisfactory. A high gloss material is best, since it leaves the animal shining as if it were moist with natural mucus. A fast drying liquid is essential, however, since the specimens must be handled and turned several times in order to get thorough coverage. Application of the liquid with a small brush proved to be rapid and effective. Use of an aerosol coating preparation (such as KRYLON Crystal Clear Spray Coating No. 1300A) is equally good, but more wasteful of material.

Mounting

Finally, the specimens can be mounted for display or study by pinning them as if they were insects. Large specimens require 2 pins to keep them from pivoting. We have found that freeze-dried slugs can be attacked and damaged by dermestid beetles just as are dried insects, but a supply of paradichlorobenzene in the box or case will protect them.

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A Device for Collecting Free-Swimming Bivalve Larvae from Laboratory Aquaria

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(1 Text figure)

It is frequently difficult to collect planktotrophic larvae from large laboratory aquaria for use in experimental work. Removing free-swimming larvae from cultures by pipette is both tedious and inefficient. It would, therefore, be useful to have a device which automatically collects and stores larvae until needed. Such an apparatus was constructed while studying the effects of temperature and reduced salinity on the larvae of the wood-boring pelecypod, *Lyrodus pedicellatus* Quatrefages (1849) (Eckelbarger & Reish, 1973). Large numbers of larvae were periodically required for use in experiments, and